Highly Efficient and Marker-free Genome Editing of Human Pluripotent Stem Cells by CRISPR-Cas9 RNP and AAV6 Donor-Mediated Homologous Recombination.

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Public Summary:
Gene editing and correction in patent-derived induced pluripotent stem cells (iPSCs) offers exciting possibilities for regenerative medicine as well as disease modeling and drug screening. However, a major challenge toward this goal is the low efficiency of gene editing using conventional methods. Here, we show that very high targeted gene editing can be achieved in human iPSCs by combining CRISPR/Cas9 nucleases with adeno-associated virus DNA repair template delivery. These new methods open up exciting new applications for iPSCs in research and medicine.

Scientific Abstract:
Genome editing of human pluripotent stem cells (hPSCs) provides powerful opportunities for in vitro disease modeling, drug discovery, and personalized stem cell-based therapeutics. Currently, only small edits can be engineered with high frequency, while larger modifications suffer from low efficiency and a resultant need for selection markers. Here, we describe marker-free genome editing in hPSCs using Cas9 ribonucleoproteins (RNPs) in combination with AAV6-mediated DNA repair template delivery. We report highly efficient and bi-allelic integration frequencies across multiple loci and hPSC lines, achieving mono-allelic editing frequencies of up to 94% at the HBB locus. Using this method, we show robust bi-allelic correction of homozygous sickle cell mutations in a patient-derived induced PSC (iPSC) line. Thus, this strategy shows significant utility for generating hPSCs with large gene integrations and/or single-nucleotide changes at high frequency and without the need for introducing selection genes, enhancing the applicability of hPSC editing for research and translational uses.